B4

15. (Amended) An isolated mammalian glycosylated monokunin, wherein the glycosylated monokunin comprises at least one sialic acid residue.

Please add new claims 25-26 as follows.

B5

25. (New) The glycosylated bikunin of claim 2, wherein the glycosylated bikunin comprises at least one N-acetylneuraminic acid residue.

26. (New) The glycosylated monokunin of claim 15, wherein the glycosylated monokunin comprises at least one N-acetylneuraminic acid residue.

Remarks

Specification

The specification has been amended at pages 3 and 13. No new matter is added with this amendment. A marked-up copy of the specification is included herewith. The paragraph at page 13, lines 3-25 has been amended to include the ATCC accession number. In addition, the paragraph at page 3, lines 12-16 has been amended to refer to U.S. Patent number 6,136,599 rather than U.S. Patent Application number 09/209,920. Withdrawal of the objections to the specification is respectfully solicited.

Claims

Claims 3-5 and 16-17 have been canceled without prejudice to the filing of continuing applications. In addition, claims 2 and 15 have been amended such that they are drawn to an isolated mammalian glycosylated bikunin (claim 2) or monokunin (claim 15), wherein the bikunin or monokunin comprises at least one sialic acid residue. Further, new claims 25 and 26

have been added and are drawn to such glycosylated bikunin (claim 25) or monokunin (claim 26) comprising at least one N-acetylneuraminic acid residue.

No new matter is added with these amendments. For example, see page 1, lines 6-8; page 4, lines 17-19; and example 3, pages 14-15 as support for the amendments made to claims 2 and 15. Support for new claims 25 and 26 is found, for instance, at page 1, lines 6-8; page 4, lines 17-19; and example 3, pages 14-15. A marked-up copy of the claims is included herewith.

Claim Rejections – 35 U.S.C. § 102

The Examiner has rejected claims 2 and 6-9 under 35 U.S.C. § 102(b) as anticipated by Kawaguchi et al. Kawaguchi merely discloses treatment of HAI-2 with N-glycosidase and subsequent analysis by SDS-PAGE. For example, see page 27560, right-hand column, first full paragraph. From this procedure, Kawaguchi concludes that HAI-2 is glycosylated. However, Kawaguchi is unable to determine anything more specific about the glycosylation of HAI-2.

In contrast, the instant application discloses and claims mammalian bikunin with a specific kind of glycosylation: sialic acid modification. For instance, see Example 3 at pages 14-15: carbohydrate analysis of bikunin produced from CHO cells. Example 3 shows the determination of the sialic acid content of bikunin, the results of which are depicted in Table 2. The applicants respectfully submit that since claim 2 specifically recites glycosylated bikunin *comprising at least one sialic acid residue* while Kawaguchi discloses HAI-2 that is only "glycosylated," the instant claims are not anticipated by Kawaguchi. Withdrawal of the anticipation rejection is respectfully requested.

Claim Rejections – 35 U.S.C. § 103

The Examiner has rejected claims 9 and 15 under 35 U.S.C. § 103(a) as unpatentable over Gentz et al. in view of Gribben et al. and Hotchkiss et al. The combination of Gentz with Gribben and Hotchkiss fail to render claims 9 and 15 nonobvious for several reasons. First, claim 9 (which depends on claim 2) is directed to bikunin *that comprises at least one sialic acid residue*. Similarly, claim 15 is directed to a monokunin *that comprises at least one sialic acid residue*. Neither Gentz nor Gribben nor Hotchkiss teaches or remotely suggests sialic acid modification of proteins. In fact, Gentz contains only a vague statement regarding *possible* glycosylation of the proteins taught therein. See page 18, lines 1-3.

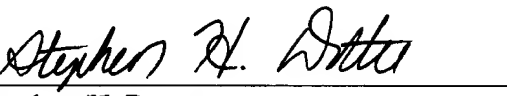
Moreover, Gribben teaches the development of antibodies to unglycosylated GM-CSF while Hotchkiss teaches the effect of glycosylation on clearance rates of rt-PA. Since Gentz concerns the isolation and characterization of TFPI-3, an entirely different protein, there is absolutely no motivation to combine Gribben and Hotchkiss with Gentz. Therefore, the applicants respectfully submit that claims 9 and 15 are nonobvious over Gentz in view of Gribben and Hotchkiss and withdrawal of the rejection is requested.

Conclusion

Allowance of the claims and passage of the case to issue are respectfully solicited. Should the Examiner believe a discussion of this matter would be helpful, she is invited to telephone the undersigned at (312) 913-0001

Respectfully submitted,

Dated: May 22, 2002



Stephen H. Docter
Reg. No. 44,659

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 South Wacker Drive
32nd Floor
Chicago, Illinois 60606
312-913-0001 (telephone)
312-913-0002 (facsimile)

Appendix A: Replacement Paragraphs in the Specification

With Markings to Show Changes Made

Page 3, lines 12-16:

This invention describes a method for the production of glycosylated placental bikunin. Preferably the cell host is CHO cells, but the production can be done with other cell hosts including HKB cells (see U.S. Pat. [Appl. Ser.] No. [09/209,920 to Cho filed December 10, 1998] 6,136,599, which is incorporated herein by reference), myeloma, and 293S cells. The production medium is preferably a chemically-defined medium free of plasma protein supplements.

Page 13, lines 3-25:

Stable production cell lines that secrete high quantities of bikunin were developed by transfecting CHO(dhfr-) cells with the expression vector shown in Figure 1. The vector was constructed using standard recombinant DNA techniques as described in U.S. Pat. No. 5,612,213 to Chan and in Sambrook et al., 1989 (supra). The expression vector contains discrete expression cassettes for the bikunin gene (truncated bikunin – amino acid sequence given in Figure 2) and the amplifiable and selectable gene DHFR (dihydrofolate reductase). About 1 x 10^6 CHO (Chinese hamster ovary) cells were transfected with 10 μg of pBC-BK using Lipofectin reagents (Life Technology, Bethesda, Maryland) according to manufacturer's instructions. The cells were then selected in the presence of 50 nM methotrexate and grown in DME/F12 media deficient in thymidine and hypoxanthine plus 5% dialyzed fetal bovine serum. Cell populations were screened for bikunin production with a chromogenic assay. Briefly, bikunin standards or culture fluid was serially diluted and incubated with an equal volume of kallikrein at 37° C for 30 minutes after which a chromogenic substrate, N-benzoyl-Pro-Phe-Arg-pNA, was added. The reaction was incubated for 15 minutes before the addition of 50% acetic acid. The amount of p-nitroanilide released was measured at 405 nM. The high producing populations were further selected in media containing increasing concentrations of methotrexate (100 to 400 nM methotrexate) and screened for the production of bikunin. Limiting dilution cloning was then applied to derive clones with high and stable productivity. The cloning was done in the absence of methotrexate using standard tissue culture techniques by depositing 1 cell/well in 96-well

plates. A clone designated FD3-1 was chosen for productivity evaluation in a bioreactor and was deposited on November 12, 1999 with the American Type Culture Collection (ATCC), Rockville, MD, and was assigned accession number [_____] PTA-94.

Appendix B: Rewritten Claims With Markings to Show Changes Made

2. (Amended) An isolated mammalian glycosylated bikunin, wherein the glycosylated bikunin comprises at least one sialic acid residue.

15. (Amended) An isolated mammalian glycosylated monokunin, wherein the glycosylated monokunin comprises at least one sialic acid residue.